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14. ABSTRACT Cytomegalovirus (CMV) induces strong and long-lasting immune responses, which make it an attractive candidate for a cancer vaccine vector. In this study, we tested whether Her2/neu expressed in CMV can induce tumor specific immune responses and mount an anti-tumor effect against breast cancer. We have constructed a MCMV-neu vaccine expressing a truncated rat Her2/neu containing extracellular and transmembrane domains. A single dose of MCMV-neu vaccination induced complete rejection of Tubo breast cancer cells in mice. We have also finished the construction of a spread deficient MCMV-neu, which lacks the gL gene required for viral entry into host cells. The project has been moving as we proposed. Next, upon the approval of the animal protocol by DOD, we will measure the immune responses induced by the vaccines and determine the effectiveness of the vaccines in animal model and determine the optimal vaccination regimen for the replication-deficient vaccine. We believe that the immune responses induced by CMV vaccine will help to drive a long lasting antitumor effect against breast cancer. In conclusion, our primary findings have suggested for the first time that CMV is a promising vector for breast cancer vaccine.					
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Introduction

Cytomegalovirus (CMV) is a beta herpes virus. After initial infection, it induces strong and long-lasting immune responses, which make it an attractive candidate for a breast cancer vaccine vector. Notably, CD8 T cells specific to some CMV epitopes can stay at a high level years after the initial infection. The goal of this proposal is to develop a crippled CMV vaccine vector expressing the HER-2/neu tumor antigen and to test both its safety and its ability to induce robust long-term ‘inflationary’ T cell memory to HER-2/neu. To achieve this goal we will initially generate a replication-competent virus (MCMV-IE-neu). The virus will be generated by mutagenesis of the MCMV genome expressed in a bacterial artificial chromosome (BAC) vector. In the meantime, we will mutate the MCMV-IE-neu BAC further to delete the gene for gL, to generate the replication deficient vaccine Δ gL-MCMV-IE-neu. Δ gL-MCMV-IE-neu will be tested for immunogenicity in comparison to MCMV-IE-neu, and vaccination protocol optimized to achieve robust long-term immunity. Both CMV vaccine strains will then be tested in their ability to provide protection against tumor challenge with the Her-2/neu expression breast cancer cell line TUBO. Finally, the safety of the Δ gL-MCMV-neu vaccine will be assessed.

Body

Task1.

We have finished the construction of MCMV-NEU vaccine. As we proposed in our proposal, we have made the BAC-CMV containing rat v-erb-b2 erythroblastic leukemia viral oncogene homolog 2 (ERBB2, also called Her2 or NEU). The reason to choose rat NEU is that allogeneic protein induces stronger immune responses in mice compared to self-antigen. Also, there is a transgenic mouse strain with rat NEU, which develops breast cancer spontaneously. Rat NEU is “self-antigen” in these mice. These mice will give us a chance to study the efficacy of CMV vaccine carrying self-antigen. The coding sequence of NEU from rat was amplified by PCR. Then, the PCR product of NEU coding sequence was inserted into vector pCMV-Kan/neo which contains a CMV promoter immediately upstream of the insert site. A kanamycin resistant gene flanked by two FRT sequences was inserted into the location downstream of NEU. The complete structure of the consequent plasmid is shown in Figure 1.

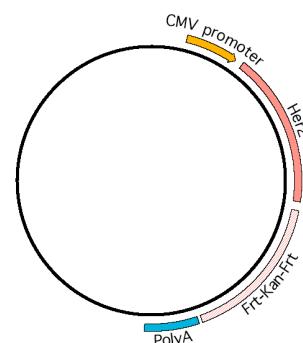
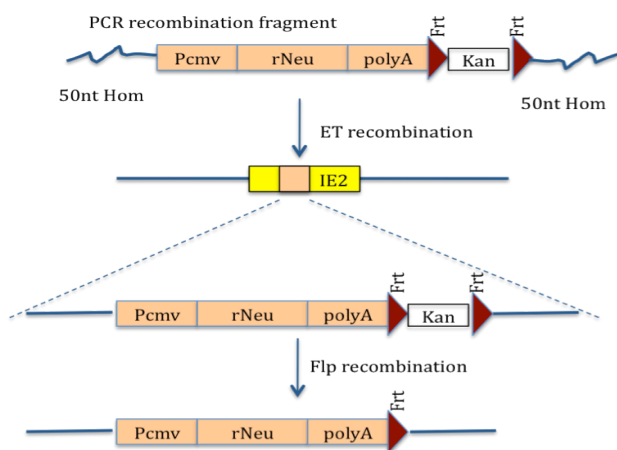


Figure 1. Map of pCMV-NEU

Fig 2 is a schematic show of the construction of MCMV-NEU BAC. We designed primers



containing overhangs homologous to the insert site in MCMV, IE2. The PCR product contained CMV promoter+Neu+ (Frt-kan-Frt) +polyA. We have

Fig 2. Rat Neu coding sequence was inserted MCMV-BAC (a bacterial artificial chromosome containing the entire genome of MCMV smith strain) at IE2 locus by E/T recombination. Consequently, the MCMV-rNEU-BAC contains the entire MCMV genome and the NEU expression cassette. Following selection of recombinant BACs on the basis of kanamycin resistance, the Kan marker was removed by flp-mediated recombination. [polyA, polyadenylation site; Kan, kanamycin resistance gene for selection in bacteria; Frt, flp-recombinase recognition site].

explained in our proposal the reason why we choose IE2 site to insert tumor antigen. It has been proved in our previous experiment that antigen inserted into IE2 position could induce strong antigen specific immune responses.

We transformed BAC-MCMV with the PCR product and get BAC-MCMV-NEU. The kanamycin gene was cleaved off by Flippase. We generated MCMV-NEU virus by transfecting 3T3 cells with the above BAC. MCMV-NEU virus was expanded and the titer of the virus was determined using plaque assay. As shown in figure 3, cells infected with MCMV-NEU expressed neu as determined by western blotting.

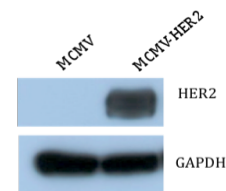


Figure 3. Expression of Her2/NEU in cells infected with MCMV-NEU. 3T3 cells were infected with MCMV-NEU for 4 days. Cells were then harvested and Her2/NEU expression was determined by western blot.

Task2.

We have preliminarily infected a few mice with MCMV-NEU and found a good antitumor effect (**Fig 4**). We will determine the antigen specific T cell response to the vaccine by flow cytometry and the antigen specific antibody response as well. We will optimize the immunization protocol according to the immune responses observed after vaccination. We just submitted our animal protocol and the approval from our IACUC to DOD for their approval. DOD funding requires us to get their approval before any further experiments with mice. Therefore, we have requested a one-year extension for this project. Upon their approval, we will continue our mouse experiments.

Task3

We have generated the replication deficient MCMV-Neu by depleting glycoprotein L(gL) gene which is involved in the entry of MCMV into target cells. To delete the gL gene in MCMV-NEU, we generated a PCR product containing an ampicillin resistant gene flanked by homologous sequences to the gL gene. Then the PCR product was inserted into MCMV-NEU at the gL gene. The resulting Δ gL-MCMV-neu BAC was transfected to NIH3T3 cells expressing complement gL gene. We are currently expanding the virus.

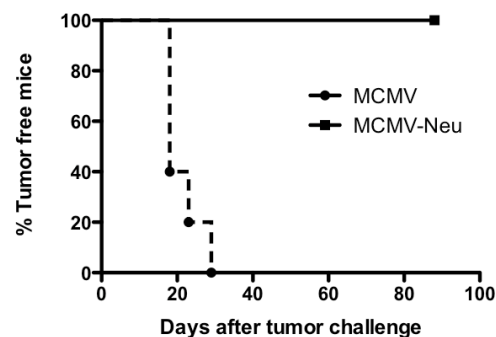


Fig 4 MCMV-NEU induced potent antitumor effect in Balb/c mice. Mice(n=5) received a single i.P. injection of MCMV-NEU 2 months prior to the challenge with Tubo cells. As a control (n=5), one group of mice were injected MCMV.

Task 4

We will test the immune responses to Δ gL-MCMV-neu and optimize the immunization to elicit the best immune responses. Upon the approval of the animal protocol by DOD, we will immediately start the experiments.

Task 5

The effectiveness of the MCMV-NEU vaccines in mouse breast cancer models will be tested as we proposed. Upon the approval of the animal protocol by DOD, we will immediately start the experiments.

Key Research Accomplishment

1. We constructed the first CMV-based breast cancer vaccine- MCMV-neu.
2. The MCMV-IE-neu has potent antitumor effect.
3. We constructed a spread deficient vaccine: Δ gL-MCMV-neu.

Reportable Outcomes

Currently N/A

Conclusion

In summary, the project has been moving as we proposed. We have finished the construction of the MCMV-NEU and Δ gL-MCMV-NEU vaccines. And we also saw potent antitumor effect by MCMV-NEU in our primary experiment. Next, upon the approval of the animal protocol by DOD, we will measure the immune responses induced by the vaccines, determine the effectiveness of the vaccines in the mouse model and determine the optimal vaccination regimen for the replication-deficient vaccine. We believe that the immune responses induced by CMV vaccine will help to drive a long lasting antitumor effect against breast cancer. Our primary findings have suggested for the first time that CMV is a promising vector for breast cancer vaccine. If further proved effective in the mice model, CMV-based cancer vaccine would be considered to be further explored for the treatment of cancer patients.